Liposomes in wool dyeing – the stability of dye–liposome systems and their application to untreated wool fibres

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The use of two different types of liposome suspensions (multilamellar vesicles, MLV, and large unilamellar vesicles, LUV) as carriers in the commercial dyeing of untreated wool with a milling acid dye is described. Liposomes prepared with egg phosphatidylcholine and containing the dye CI Acid Blue 90 were used. The physico-chemical stability of liposomes was studied by measuring the mean particle size distribution of phospholipidic vesicles during dyeing. The possible hydrolysis of phospholipid molecules was also determined. Kinetic aspects involving dye adsorption and bonding were investigated. Dye exhaustion on untreated wool fibres was inhibited and dye bonding was improved. The lipid concentration and type of liposomes were important factors in this process.

INTRODUCTION

Membrane phospholipids are molecules that contain a hydrophilic and a hydrophobic moiety. Their hydrophilic unit, also called the polar head group, is formed by the phosphate and choline groups, while their hydrophobic moiety is formed by two hydrocarbon chains or tails (Figure 1). These structures have aroused a great deal of interest as they provide an excellent model for cell membranes [3,4], and have potential in controlled delivery systems for therapeutic agents [5–7]. These vesicles are currently the most effective carrier for the transport and introduction of various agents into cells in *in vitro* experiments for both hydrophilic and lipophilic chemicals. Thus Barni reported that the use of double-chain surfactants suitable for the preparation of synthetic vesicles in the dyeing of polyester with disperse dyes resulted in good levelling and faster dye migration [8]. Liposomes have also been investigated for use in wool chlorination [9].

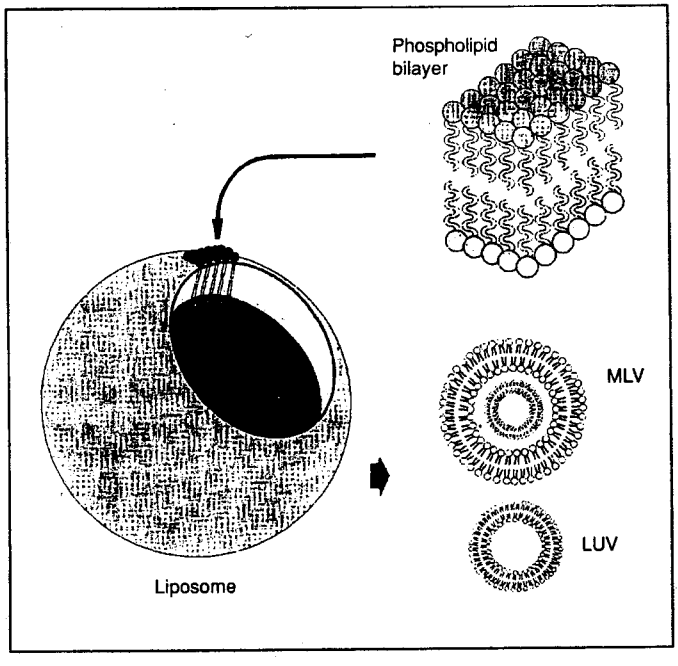


Figure 1 Liposome structures, including multilamellar vesicles (MLV) and large unilamellar vesicles (LUV)

The commercial milling acid dye Polar Blue G (CGY, CI Acid Blue 90) has high wet fastness but exhibits much slower diffusion than is typical of levelling acid dyes. However, its migration and coverage properties are inferior and normally the addition of a levelling agent is necessary [2]. Therefore it is possible that liposomes could be used as carriers for such a dye, providing improved adsorption and bonding on untreated wool fibres.

In the present study the physico-chemical stability of multilamellar and large unilamellar vesicle suspensions (MLV and LUV) of liposomes (Figure 1) containing Polar Blue G milling acid dye was investigated, at phospholipid concentrations from 0.5 to 4 mmol/l. The application of these structures in dyeing of untreated wool has also been examined, focusing on kinetic aspects of dye adsorption and the dye–fibre bonding forces on wool fibres.

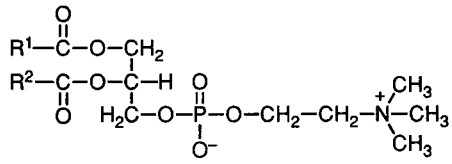
EXPERIMENTAL

Materials

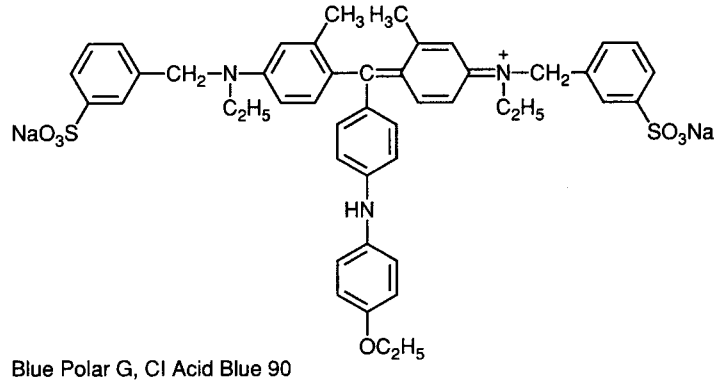
Botany wool fabrics were used, knitted from R64/2 tex (count 2/28) yarns. Samples were Soxhlet extracted for 2 h with methylene chloride and rinsed with water purified by the Milli-Ro system (Millipore) and dried at room temperature.

The structure of CI Acid Blue 90 is given in Figure 2. Phosphatidylcholine (PC) was purified from egg lecithin (Merck) according to the method of Singleton [10] and shown to be pure by thin-layer chromatography (TLC) (chemical structure also given in Figure 2).

Polycarbonate membranes of 400 and 800 nm thickness, and membrane holders used for liposome extrusion, were purchased from Nucleopore (Pleasanton, USA). Triton



Phosphatidylcholine



Blue Polar G, CI Acid Blue 90

Figure 2 Chemical structure of Polar Blue G dye (CI Acid Blue 90) and phosphatidylcholine

X-100 (octylphenol with ten units of ethylene oxide and active matter of 100%) was specially prepared by Tenneco España SA.

Preparation of liposomes

Multilamellar vesicle liposomes of defined vesicle size (400 nm) and different phospholipid concentrations (0.5 to 4 mmol/l) containing Polar Blue G were prepared following a method described by Bangham [11]. A lipid film was formed by removing the organic solvent from a chloroform solution of egg phosphatidylcholine by rotary evaporation in a nitrogen atmosphere and under vacuum (0.46 atm, 47 kPa). An aqueous phase containing the dyebath components (dye 1%, sodium sulphate 5%, acetic acid to pH 5.5) to be introduced into the liposomes was then added to the lipid film. The solutions were swirled by hand to transfer the lipid from the walls of the flask and to disperse large lipid aggregates; glass beads were added to facilitate dispersion. The resulting milky suspensions were centrifuged for 5 min. The liposome suspensions were extruded through 400 and 800 nm polycarbonate membranes to obtain uniform size distributions [12].

Large unilamellar vesicle suspensions of a defined size (400 nm) in the same range of phospholipid concentrations were prepared by reverse-phase evaporation, in a nitrogen atmosphere, as described by Paternostre and Rigaud [13,14]. This procedure was derived from the general method of Szoka and Papahadjopoulos [15]. A lipid film previously formed was redissolved in diethyl ether and an aqueous phase containing the dyebath components was added to the phospholipid solution. The resulting two-phase system was sonicated (Labsonic 1510, Braun) at 70 W for 3 min at 5°C, thereby obtaining an emulsion. The organic solvent was removed at 20°C by rotary evaporation under vacuum (0.52 atm, 53 kPa). As most of the solvent was removed, the material first formed a viscous gel before turning into an aqueous solution. Likewise, the vesicle suspensions were extruded (400 nm) to obtain a defined, uniform size distribution [12].

Dyeing procedure

Knitted wool samples, 1 g were treated with MLV and LUV liposome suspensions freshly prepared at phospholipid concentrations in the range 0.5–4.0 mmol/l in the presence of Polar Blue G. The dye was applied at 1% o.w.f., with 5% o.w.f. anhydrous sodium sulphate. Dyeing was started at 50°C and the temperature raised by 0.9 degC/min to 90°C. Dyeing was continued for 120 at a liquor ratio of 60:1 and pH 5.5. Afterwards samples were rinsed with water for 10 min and dried at room temperature. Laboratory dyeing was carried out in a Multi-Mat dyeing machine (Renigal).

Dyebath exhaustion was determined spectrophotometrically using a Shimadzu UV-265FW spectrophotometer. Liposome aliquots (0.5 ml) were periodically added to quartz cuvettes filled with 2 ml of aqueous solution of Triton X-100 (10 g/l), supplemented by sodium sulphate (5%) and acetic acid at pH 5.5. The interaction between the nonionic surfactant Triton X-100 and liposome structures resulted in a solubilisation of phospholipid vesicles via mixed micelle formation [19,20], turning the liposome suspensions into clear solutions.

Figure 3 shows the effect of the cleavage of liposome vesicles by Triton X-100 on the absorption spectra of Polar Blue G at the highest phospholipid concentration (4 mmol/l). It can be seen that the wavelength of maximum absorption of the dye used in this research does not change in the presence of phospholipid–surfactant mixed micelles.

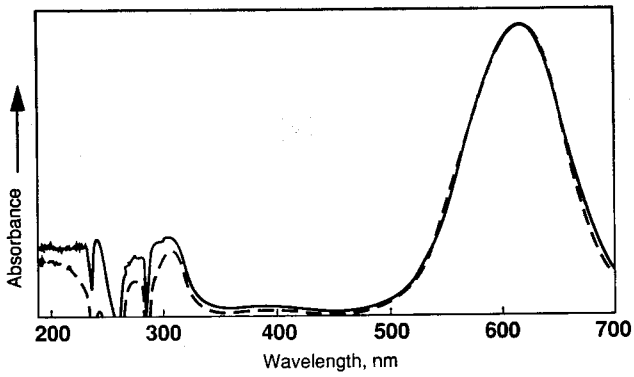


Figure 3 Absorption spectra of Polar Blue G in the presence (discontinuous line) and absence (continuous line) of (phospholipid/Triton X-100) mixed micelles

Determination of the encapsulation efficiency of liposomes

The amount of encapsulated dye in liposomes (expressed in terms of percentage volume) was determined by a spectrophotometric method. Phospholipid vesicles were first cleared of unencapsulated dye by separation through Sephadex G-50 medium resin (Pharmacia, Sweden) column chromatography and then the dye concentration was evaluated by spectrophotometry after the destruction of the liposomes by addition of Triton X-100 [21,22].

Determination of the mean size of liposome vesicles

The mean vesicle size and polydispersity of MLV and LUV suspensions was determined by a Photon correlator spectrometer (Malvern Autosizer IIC). Samples were adjusted to the appropriate concentration range. The measurements were made at 20°C, with a detection angle of 90°.

Aggregation measurements

The aggregation state of the vesicles was estimated as a measure of the physical stability of the liposome suspensions. This was done by monitoring the variation of the mean vesicle size distribution of liposome suspensions as a function of time.

Estimation of phosphorus in liposomes

The phospholipid concentration of liposomes was determined by the ascorbic acid spectrophotometric method for total phosphorus estimation [16].

Determination of fatty acid composition

The hydrolysis level of the phospholipid hydrocarbon chain was estimated as a measure of the chemical stability of liposomes. The liposome preparation was extracted three times with hexane, the organic extracts were dried with magnesium sulphate, and the lipid content was obtained after solvent removal at reduced pressure.

Fatty acids were quantitatively determined as methyl ester derivatives [17], in a Hewlett Packard 5840A gas chromatograph, equipped with a flame ionisation detector, using an internal standard of heptadecanoic acid (Fluka 51610). Methyl esters of fatty acids were prepared using the diazomethane method [18].

A 12 m long by 2 mm i.d. fused silica capillary column was used, which had been coated with methyl silicone. The operating conditions were as follows: programming temperatures T_1 100°C and T_2 200°C, heating rate 8 degC/min, detector and injection temperatures 250 and 230°C, helium carrier gas, flow rate 1 ml/min and chart speed 0.5 cm/min. The quantitative peak estimation was made by electronic integration, and the area ratios were calculated and multiplied by the response factor for each fatty acid methyl ester.

Dye extraction

The superficial dye bonded to the fibres by nonpolar forces (hydrophobic interactions, van der Waals forces and hydrogen bonds) was extracted with pure ethanol at 25°C for 60 min [23]. Subsequent extractions with ammonia (0.5% for 15 min at 60°C) stripped the dye diffused inside the fibre and bonded ionically [1].

Smoothness of dyed samples

The surface smoothness of samples treated with dye–liposome systems was determined by measuring the dynamic coefficient of friction using an Instron Textile Tester 1122 with a device designed to meet the requirements of the American Society for Testing and Materials Standards (ASTM) [24].

The sample was attached to the rubber-covered surface of a 200 g sled which was drawn across a metallic table. The sled was connected through a low friction pulley to the Instron load cell, which detected the friction strength. The moving crosshead supplied the motive force to the sled. The measurements were taken at slip rate levels of 1 and 10 mm/min.

RESULTS

Stability of liposome suspensions

The possible aggregation of liposomes during the dyeing process was monitored by measuring the variations in mean vesicle size distribution of these suspensions, using a quasielastic light scattering method [25]. The results obtained for both MLV and LUV suspensions (phospholipid concentration 4 mmol/l) are given in Table 1. It may be observed that a small increase occurs during dyeing, the polydispersity indices remaining below 0.15 after treatment. Mean vesicle size was maintained at around 400 nm and polydispersity indices were below 0.20 for up to 24 h for both liposome structures.

A sequential analysis of fatty acid composition of phospholipids using gas liquid chromatography was carried out in order to determine the changes in concentration of intact fatty acids of liposome suspensions during dyeing. The results showed that in these liposome suspensions, using heptadecanoic acid as an internal standard, no hydrolysis of the ester bond in the phospholipid molecule took place.

Table 1 Mean particle size distribution of LUV and MLV liposome suspensions (4 mmol/l phospholipid concn) during dyeing with Polar Blue G

Time (min)	Mean vesicle size (nm)		Polydispersity index	
	LUV	MLV	LUV	MLV
0	400	404	0.092	0.097
5	402	405	0.092	0.097
10	398	400	0.094	0.096
15	396	390	0.120	0.113
30	402	407	0.123	0.119
60	420	426	0.132	0.126
90	426	430	0.142	0.135
120	434	438	0.150	0.149

Encapsulation efficiency of liposomes

The amounts of encapsulated dye in MLV and LUV suspensions are given in Figure 4. LUVs showed a higher encapsulation efficiency (around 25% for 4.0 mmol/l lipid concentration), whereas MLVs had values around 15% for identical lipid concentrations). The encapsulation efficiency increased in direct proportion with lipid concentration of liposomes for both structures studied.

The higher encapsulation efficiency of the LUV suspensions could be explained by the unicompartmental architecture of these species, whose vesicles show a higher internal volume [21].

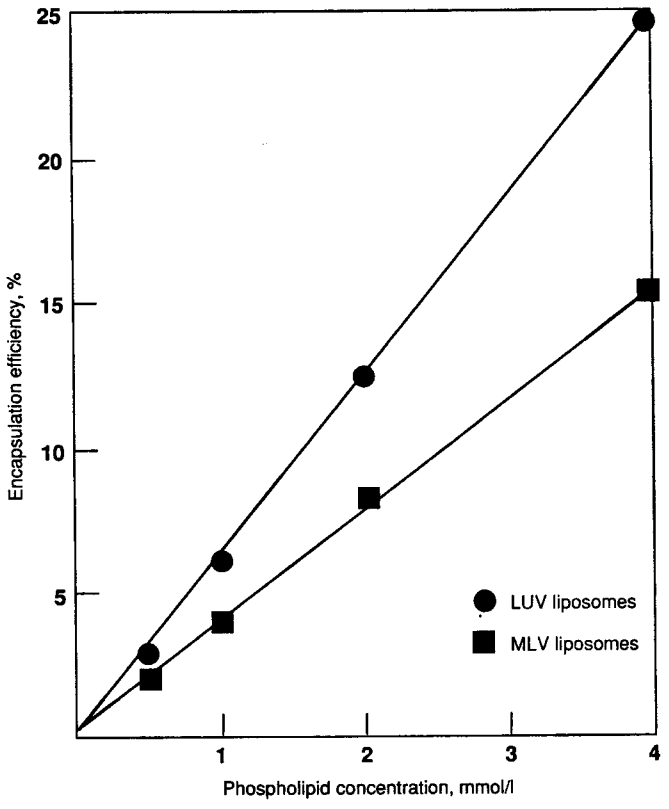


Figure 4 Percentage encapsulation efficiency of LUV and MLV liposome suspensions containing Polar Blue G at different lipid concentrations

Dyeing kinetics

The results of measuring dye exhaustion are plotted in Figure 5 (A for LUV and B for MLV). The use of liposomes in dyeing results in an inhibition of dye exhaustion for both structures studied, the effect being slightly greater for LUV suspensions (Figure 5A). This behaviour seems to be closely connected with the lipid concentration of liposomes, reaching the highest point for 4.0 mmol/l and remaining constant throughout the dyeing process. The final dye exhaustion for 4 mmol/l lipid concentration was approximately 77% for LUV and 83% for MLV liposome suspensions.

Influence of liposomes on bonding of dyes on wool

In order to find out whether liposomes as dye carriers caused changes to dye–fibre bonding forces after dyeing, extractions in pure ethanol [23] and ammonia [1] were performed on the dyed samples. The results are given in Table 2. The amounts of extracted dye were closely related to the lipid concentration for both structures used. Ethanol extractions (at 4 mmol/l lipid concentration) showed values less than 0.1 mg dye per gram wool, whereas similar extractions of samples dyed in the absence of liposomes had notably higher values (0.2–0.3 mg dye per g wool). Similar tendencies could be observed in subsequent extractions on the same samples using ammonia, although the amounts of extracted dye were lower in samples dyed with LUV liposomes.

Table 2 Amounts of dye extracted after dyeing untreated wool via LUV and MLV liposome suspensions at different lipid concentrations

Lipid concn (mmol/l)	Amount of dye extracted (mg dye/g wool)			
	LUV		MLV	
	A	B	A	B
4.0	0.012	1.198	0.022	1.798
2.0	0.022	1.906	0.025	2.200
1.0	0.034	2.084	0.040	2.400
0.5	0.049	2.983	0.048	3.020
0	0.217	3.608	0.200	3.620

A – Extraction with pure ethanol [23]
B – Extraction with ammonia [1]

The total percentage of dye bonded to the wool fibres can be expressed by Eqn 1:

$$C_b = \frac{C_a - C_e}{C_a} \times 100 \qquad (1)$$

where C_b is the amount of dye bonded (%), C_a is the amount of dye absorbed (mg per g wool) and C_e is the amount of dye extracted (mg per g wool).

Table 3 gives the total percentages of bonded dye

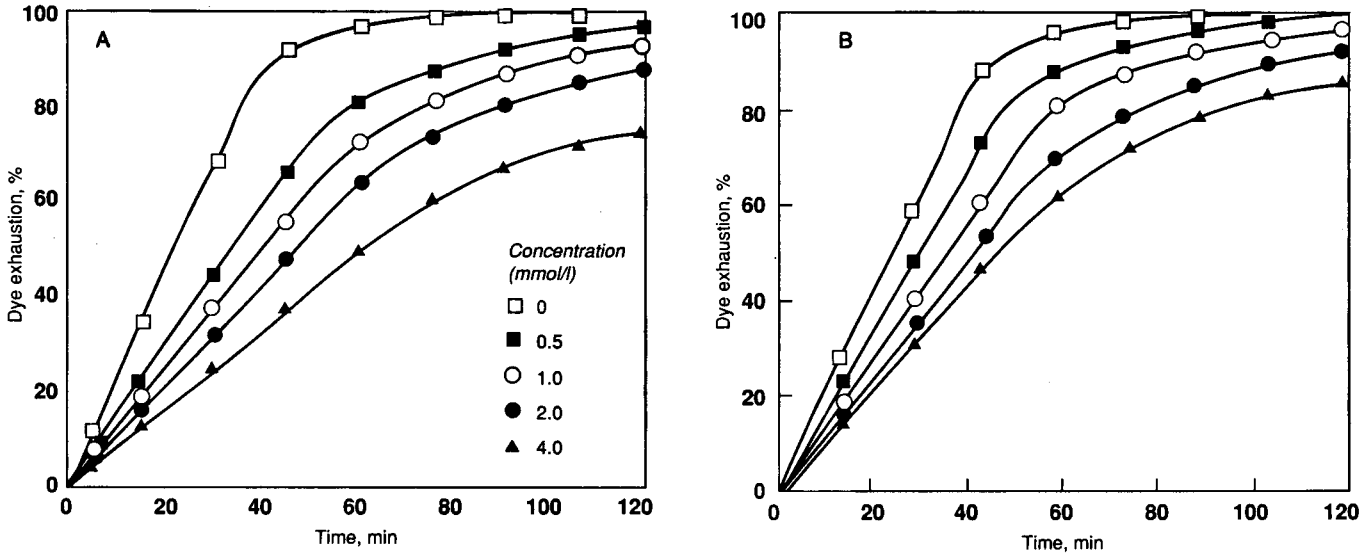


Figure 5 Exhaustion of Polar Blue G on untreated wool samples in dyeing via LUV (A) and MLV (B) liposomes

Table 3 Amounts of total dye bonded using LUV and MLV liposomes at different lipid concentrations with Polar Blue G

	Total bonded dye (%)	
Lipid concn (mmol/l)	LUV	MLV
4.0	84.13	77.25
2.0	78.08	73.82
1.0	77.12	72.88
0.5	68.06	67.70
0	62.16	61.80

(derived from Eqn 1) after dyeing wool samples using LUV and MLV liposomes. The use of MLV liposomes results in all cases in low percentages of total bonded dye compared with levels found with LUV liposomes. The improved percentages of bonded dye using LUV liposomes are the result of the optimum balance between the amounts of absorbed dye during the dyeing process and the total extracted dye. These improvements may be attributed to the structural characteristics of these liposomes whose vesicles show a higher encapsulation efficiency (Figure 4).

Figure 6 shows the amounts of bonded dye in wool fibres given as a difference between the amounts of adsorbed dye and total extracted dye versus phospholipid concentration. A maximum amount of bonded dye was obtained with a lipid concentration of 1 mmol/l for both liposome structures used.

Since pure ethanol extractions remove the dye superficially bonded to the fibres by nonpolar forces, the presence of liposomes in dyeing must lead to a decrease in such dye. Likewise, the decreasing amounts of dye removed with ammonia with respect to the lipid concentration of liposomes could be attributed to the increasing contribution of the nonpolar forces in the dye–fibre bonds. These interactions, especially those that are hydrophobic in nature, may play an important role in the dye–fibre bonding inside the fibre.

Smoothness of dyed samples

The smoothness of dyed samples expressed in terms of friction strength between samples and a metallic flat surface are given in Figure 7. The friction strength slightly decreased as the lipid concentration of liposomes in the dyebath increased. The best results were obtained using 1–2 mmol/l phospholipid concentrations. This trend was similar for both liposome structures studied.

Further work

After the results of the present work have been analysed, some questions remain to be answered, such as: how do phospholipid bilayers interact with the hydrophobic

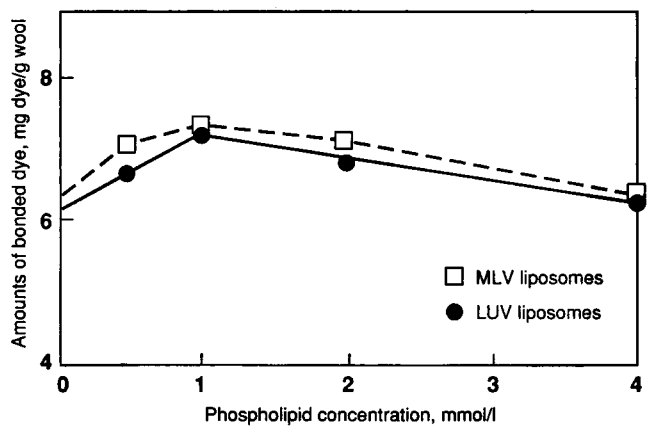


Figure 6 Total amounts of dye bonded expressed as a difference between dye absorbed (Figure 3) and total extracted dye, versus phospholipid concentration of LUV and MLV liposomes for Polar Blue G

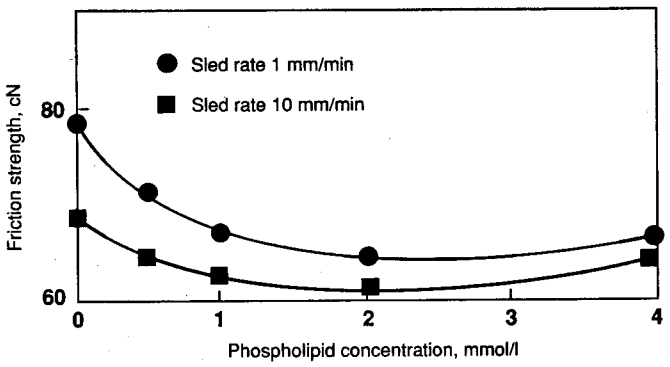


Figure 7 Smoothness of dyed samples expressed in terms of friction strength between samples and a metallic surface versus lipid concentration in liposomes

groups of wool? and how does this interaction affect the adsorption and bonding of dyes on wool fibres? An attempt to answer these questions will be the subject of future studies.

CONCLUSIONS

From our findings, it seems that a new method of wool dyeing via LUV and MLV liposomes could be considered suitable for the control of dye exhaustion using milling acid dyes.

LUV and MLV liposome suspensions at pH 5.5 are physico-chemically stable during the dyeing process at phospholipid concentrations ranging from 0.5 to 4.0 mmol/l for up to 24 h following preparation.

The inhibition of dye exhaustion on untreated wool fibres is directly dependent on both the phospholipid concentration and the structure of liposomes. Thus the use of LUV liposome structures results in a greater decrease in dye exhaustion compared with MLV liposomes. The maximum inhibition corresponds to the highest lipid concentrations for both liposome structures studied.

The use of LUV liposomes results in higher percentages of total bonded dye compared with those with MLV liposomes. The maximum amount of bonded dye was obtained at 1 mmol/l phospholipid concentration for both liposome structures, which corresponds to approximately 45 mg of phospholipid per gram of wool (4.5% o.w.f.).

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REFERENCES

1. E R Trotman, *Dyeing and chemical technology of textile fibres* (High Wycombe, Bucks.: Charles Griffin, 1984), chapter 12.
2. J A Bone, J Shore and J Park, *J.S.D.C.*, **104** (1988) 12.
3. A de la Maza *et al.*, *J. Amer. Oil Chem. Soc.*, **68** (1991) 315.
4. K Mohr and M Struve, *Biochem. Pharmacol.*, **41** (1991) 961.
5. D Chapman, *Liposome technology*, Vol. 1, Ed. G Gregoriadis (Boca Raton, Florida: CRC, 1986) 1.
6. E Mayhew and D Papahadjopoulos in *Liposomes*, Ed. J M Ostro (New York: Marcel Dekker, 1983).
7. F Szoka and F D Papahadjopoulos in *Liposomes: from physical structure to therapeutic applications*, Ed. C G Knight (Amsterdam: Elsevier, 1981).
8. E Barni *et al.*, *J. Disper. Sci. Technol.*, **9** (1988) 75.
9. A de la Maza, J L Parra and P Bosch, *Text. Research J.*, **61** (1991) 357.
10. W S Singleton *et al.*, *J. Amer. Oil Chem. Soc.*, **42** (1965) 53.
11. A D Bangham, M M Standish and J C Watkins, *J. Mol. Biol.*, **13** (1965) 238.
12. F Szoka *et al.*, *Biochim. Biophys. Acta*, **601** (1980) 559.
13. J L Rigaud, A Bluzat and S Buschlen, *Biochem. Biophys. Res. Commun.*, **111** (1983) 373.
14. M T Paternostre, M Roux and J L Rigaud, *Biochem.*, **27** (1988) 2668.
15. F Szoka and D Papahadjopoulos, *Proc. Nat. Acad. Sci. USA*, **75** (1978) 4194.
16. M C Rand, A E Greenberg and M J Taras, *Standard methods for the examination of water and wastewater*, 466 (Washington DC: Amer. Public Health Assocn, 1976).
17. W W Christie, *Gas chromatography and lipids* (Ayr, Scotland: The Oily Press, 1989).
18. T J de Boer and H J Backer, *Org. Synth. Coll.*, **4** (1963) 250.
19. A Hellenius and K Simons, *Biochim. Biophys. Acta*, **415** (1975) 29.
20. B R Lichtenberg, J Robson and E A Dennis, *Biochim. Biophys. Acta*, **737** (1983) 285.
21. D W Deamer and P S Uster in *Liposomes*, Ed. J M Ostro (New York: Marcel Dekker, 1983).
22. J N Weinstein *et al.* in *Liposome technology*, Vol. 3, Ed. G Gregoriadis (Boca Raton, Florida: CRC, 1986) 183.
23. J Sanchez Leal and J J Garcia Dominguez, III Premio Jorge Camp. (1973).
24. Coefficients of friction of surfaces, Part 48, ASTM D 1894-78. (Philadelphia: ASTM, 1980).
25. C S Chong and K Colbow, *Biochim. Biophys. Acta*, **436** (1976) 260.